Genomics of Paratuberculosis and Tuberculosis in Livestock

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Outline of the presentation

- **Macrosys EC**

ParatB cattle

- **Episud (Italian PON project)**

TB and ParatB in livestock (pigs, sheep, goats)

- **Micromap (Italian PON projects)**

ParaTB, MAP whole genome sequencing

Macrosys

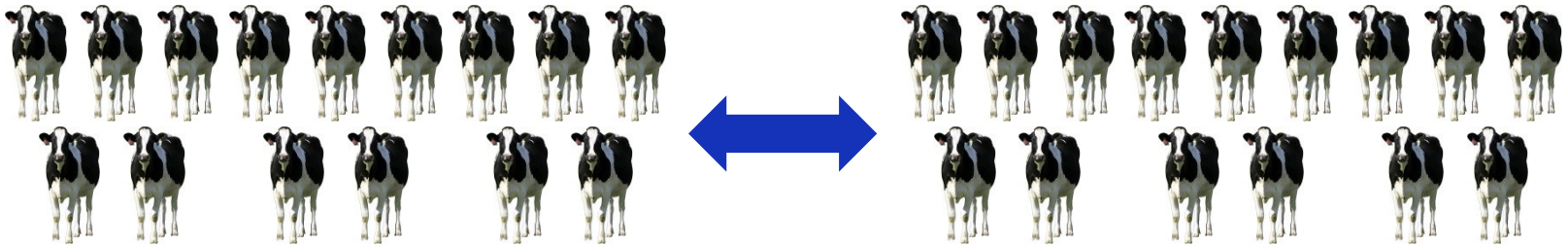
Johne's Disease

- Mycobacterium avium Ssp.Paratubercolosis (MAP) causes Johne's disease

- Chronic enteritis characterized :
  - diarrhea
  - weight loss
  - drop in milk production
  - Death

- Not treatable, vaccine efficacy controversial
- Host genetic component

Perform a case-control genome wide scan in Holstein cattle using the Bovine50KSNPChip



500 cases **500 controls**

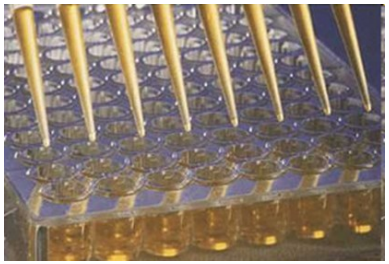
Localize regions and genes having an impact on Johne's disease susceptibility

Selection criteria

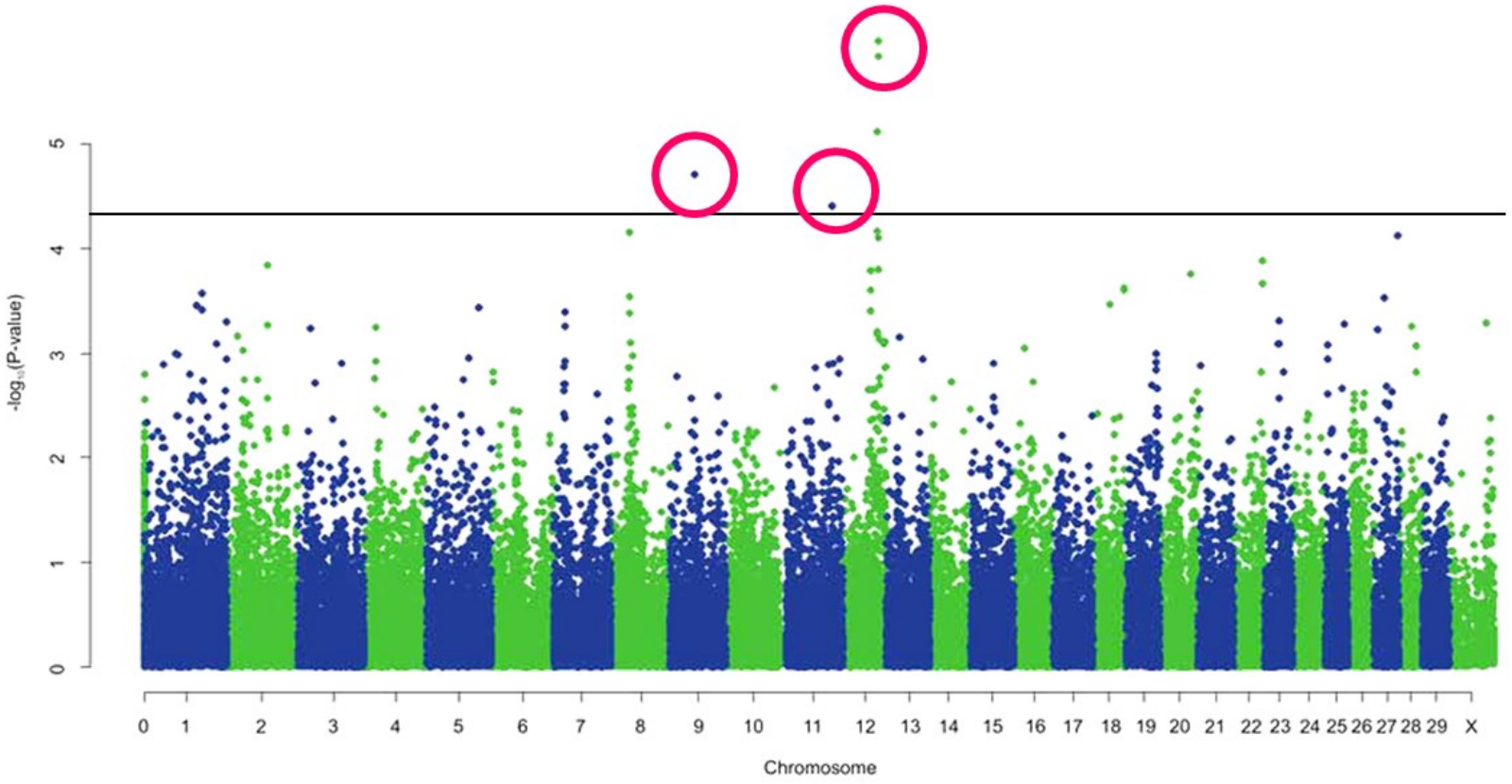
- Over 5000 samples collected 2007-2008
- Status based on ELISA test MAP

Total samples	ELISA negative	ELISA positive
991	495	496

- Holstein Cattle Females
- Use as many herds as possible (120)
- 2 positive and 2 negative animals per herd
- Age, sex, herd matched, test day matched



Results GWAs



Significance association to Johne's disease on BTA 9, 11 and 12

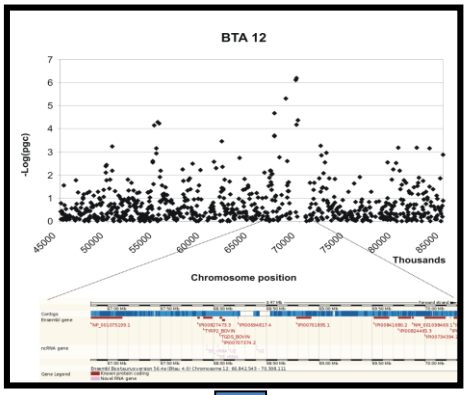
Minozzi et al. PlosOne 2010

Results 1. TOP SNPs

SNP name	BTA	Position (bp)	p-value
ARS-BFGL-NGS-57278	12	69.663.832	1.04e-06
BTA-95991-no-rs	12	69.599.639	1.44e-06
ARS-BFGL-NGS-101584	12	68.553.182	7.70e-06
ARS-BFGL-NGS-8531	9	46.362.363	1.94e-05
ARS-BFGL-NGS-17731	11	89.695.127	3.93e-05
ARS-BFGL-NGS-105846	12	67.342.543	6.83e-05
BTB-02056135	8	37.257.076	7.02e-05
ARS-BFGL-NGS-37647	27	45.253.563	7.55e-05
BTB-01470661	12	69.808.111	7.80e-05

Gene in candidate regions and network - pathway analysis

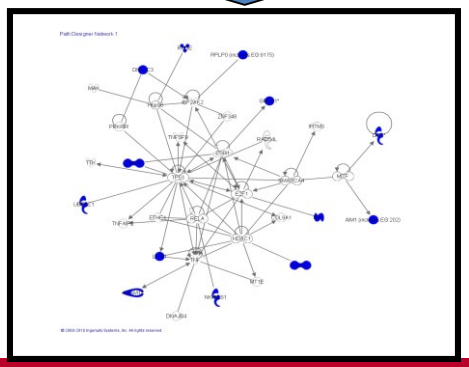
- Genes within upstream and downstream the 6 most significantly associated SNP



- Blast to perform a homology search against the Human RefSeq and UniProt databases

BLAST

- Ingenuity Network and Pathway Analysis



Results. Network Analysis

	Top Functions	Score	Gene	Molecules in Network
1.	Antimicrobial Response Infection Mechanism Cellular Growth and Proliferation	27	13/35	**ADAM17,AIM1**,COL9A1,**DCT,DNAJC3,** DNAJB4,**E2F1**,**EIF2**AK2,ESR1,ETHE1,**GREB1**,HDAC1,HSP90,IFITM3,**KLF11,LPIN1**,MAK,MITF,MT1E,**NKIRASI**,PRKRIR,RAD54L,**RAGE**,RELA,**RPLPO,** SMARCA4, **TAF1B**, TNF, TNFAIP2, TNFSF9, TP53, TTK, **UBE2E1**, ZNF346
2.	Nucleic Acid Metabolism Small Molecule Biochemistry Cell Morphology	26	12/36	ALPF72, ARHGEF2, C160RLF, CKC2, **CLDN10**, CPD, **EEF1A1**, ERAP1, EZH2, **GPR180, HIGDIA**, MIR103-1, MIR147, MYC, **ODC1,** PIK3R1, **PRDMI,** RFX3, **ROCK2, PRL15,** RPL27, RPL21, RRAD, **RRM2**, PPM2B, RSAD2, SH2D2A, **SOX21,** SPIB, **TGDS**, THY1, **YWHAQ**, ZNF207, ZNF281
3.	Cell Death, Cellular Development Nervous System Development	23	11/34	**ATG5,** ATP5G1, ATP5H, ATP5J, ATP5J2, **ATP50**, ATP6P1, ATP6VOD2,ATP6VE2, ATP6V1B1,ATP6V1C1**, ATP6V1C2,** ATP6V1D, ATP6V1E2, ATP6V1F, ATP6V1G3, DLG4, ERLIN1, GM1335, **GPC6, GRHL1,** ATPH2S, HNF4A, HNRNPC, **HPCAL1,** MSRB2, NUDT11, **PD1A6, PREP, QRSL1,** RDG1563463, RDG1565438, **RNU2-1, RTNAIP1,** TRAF6

Results. 2 Network Analysis

	Top Functions	Score	Gene
1.	Antimicrobial Response Infection Mechanism Cellular Growth and Proliferation	27	13/35
2.	Nucleic Acid Metabolism Small Molecule Biochemistry Cell Morphology	26	12/36
3.	Cell Death, Cellular Development Nervous System Development	23	11/34

High degree of interconnection between genes (13, 12, 11)

All chromosomal regions identified by GWAs involved

Joint -Analysis

Combined raw data from two studies to identify loci associated with Johne's disease

A whole genome association analysis identifies loci associated with *Mycobacterium avium* subsp. *paratuberculosis* infection status in US holstein cattle

M. Settles*, R. Zanella*, S. D. McKay†, R. D. Schnabel†, J. F. Taylor†, R. Whitlock‡, Y. Schukken§, J. S. Van Kessel¶, J. M. Smith** and H. Neibergs*

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PLoS one

Genetic Loci Involved in Antibody Response to *Mycobacterium avium ssp. paratuberculosis* in Cattle

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Top SNPs

SNP	CHR	Position	n	p-value
ARS-BFGL-NGS-57278	12	69663832	998	1.91 e-05
BTB-01626215	0	0	998	2.49 e-05
BTA-95991-no-rs	12	69599639	1017	2.70 e-05
ARS-BFGL-NGS-36801	15	66161046	1017	2.88 e-05
ARS-BFGL-NGS-97925	1	113617698	1017	3.14 e-05
ARS-BFGL-NGS-61822	1	113855358	1017	3.70 e-05
ARS-BFGL-NGS-16218	1	114460243	1015	6.41 e-05

G. Minozzi, J.L. Williams, A. Stella, F. Strozzi, M. Luini, M.L. Settles, J.F. Taylor, R.H. Whitlock, R. Zanella, H.L. Neibergs (2012). Meta-analysis of two genome-wide association studies of bovine paratuberculosis. PLOS ONE, e32578, ISSN: 1932-6203, doi: 10.1371/journal.pone.0032578

Further outcomes of the project

- **Random Forest analysis of GWAs ParaTB data**

G. Minozzi, A. Pedretti, S. Biffani, E.L. Nicolazzi, A. Stella (2014). Genome wide association analysis of the 16th QTL- MAS Workshop dataset using the Random Forest machine learning approach. BMC PROCEEDINGS, vol. 8, p. 1-6, ISSN: 1753-6561, doi: 10.1186/1753-6561-8-S5-S4

- **GSEA analysis of GWAs ParaTB data**

M. Del Corvo, M. Luini, A. Stella, G. Pagnacco, P. Ajmone-Marsan, J.L. Williams, G. Minozzi (2017). Identification of additional loci associated with antibody response to Mycobacterium avium ssp. Paratuberculosis in cattle by GSEA SNP analysis. MAMMALIAN GENOME, p. 1-8, ISSN: 0938-8990, doi: 10.1007/s00335-017-9714-7

- **RNAseq and microRNAseq Analysis of Cattle related to ParaTB infectious status**

M. Malvisi, F. Palazzo, N. Morandi, B. Lazzari, J.L. Williams, G. Pagnacco, G. Minozzi (2016). Responses of bovine innate immunity to mycobacterium avium subsp : Paratuberculosis infection revealed by changes in gene expression and levels of MicroRNA. PLOS ONE, vol. 11, e0164461, ISSN: 1932-6203, doi: 10.1371/journal.pone.0164461

EPISUD PON Project

Program to develop tools for the identification and control of micobacterial infections in livestock.

"The aim of this project is the discovery and characterization of biomarkers that will be used to develop innovative kits for the precocious diagnosis of pathologies with high economical impact, as tuberculosis (TB) in swine and paratuberculosis (ParaTB) in sheep and goats"

RNA seq (ongoing) /proteomic

Species/Breed	TB/ParaTB	PP (n)	NP	NN
Sheep (breed COMISANA)	paratB	5	4	4
Sheep (breed VALLE DEL BELICE)	paraTB	5	5	4
Goats (breed GARGANICA)	paraTB	5	6	4
Pigs (breed Nero dei Nebrodi)	TB	4	3	4

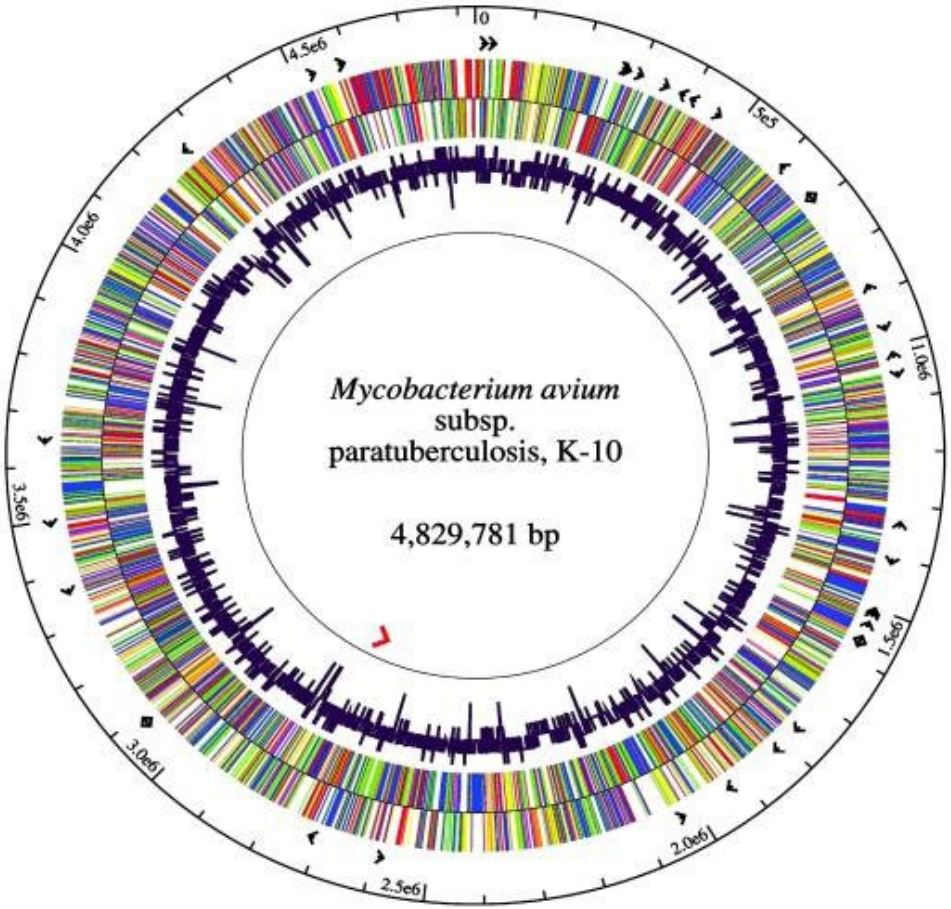
microRNA seq (ongoing)

Species/Breed		PP (n)	NP	NN
Sheep (breed COMISANA)	paratB	4	4	4
Sheep (breed VALLE DEL BELICE)	paraTB	4	5	4
Goats (breed GARGANICA)	paraTB	5	4	4
Pigs (breed Nero dei Nebrodi)	TB	5	5	4

Ongoing/Genome Wide Association Studies

Species/Breed	TB/ParaTB	PP (n)	NP	NN
Sheep (breed COMISANA)	paratB	400	400	0
Sheep (breed VALLE DEL BELICE)	paraTB	400	400	0
Goats (breed GARGANICA)	paraTB	300	300	0
Pigs (breed Nero dei Nebrodi)	TB	180	180	0

MICROMAP project



Bovine MAP

ongoing

- Illumina whole genome sequencing at 30X coverage

- Sequence 70 Italian isolates to associate genomic varibility and virulence and localization

Thank you

Parco Tecnologico Padano, Italia

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Andrea Pedretti

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